AMENDMENTS TO THE DRAWINGS:

Please substitute Figures 1 and 2 with the replacement sheets filed with this paper.

REMARKS

Status of Claims

Support for the replacement sheets of Figures 1 and 2 is found in Examples 5-7 of the specification at pages 38-41. Support for amended claims 1, 4, 5, 10, 11, 13, and 41 is found in the original claims and in the specification at page 10, line 20 through page 16, line 27, Figure 2, and Examples 2-4. Support for new claims 42-50 is found in the specification at page 7, lines 23-25, page 9, line 14 through page 10, line 17, and Examples 6 and 7. Upon entry of the amendment, claims 1-6, 10-16, are 40-50 are present and active in the application. Claims 7-9 and 17-39 are canceled without prejudice to their prosecution in any divisional or continuation application. No new matter has been added as a result of the amendments.

Introduction

A hallmark of cancer cells is not only uncontrolled proliferation, but also a decreased rate of apoptosis. This attribute can confound treatments that induce apoptotic pathways to kill cancer cells. For example, a common cause of leukemia treatment failure is the development of chemotherapy-resistant disease; this drug-resistant phenotype often correlates with molecular defects in the apoptotic cellular pathways. Elucidation of the mechanisms controlling apoptosis induction and subsequent cellular disintegration would result in improved methods for the diagnosis of chemotherapy-resistant cancers.

When a cell undergoes apoptosis, the structure of the cell breaks down. The breakdown components are packaged into apoptotic bodies: membrane bound "sacs" that contain nucleic acids, proteins and lipids. Usually, macrophages or neighboring cells engulf these bodies, clearing them from the system. However, when the ability of neighboring cells and/or macrophages to engulf these bodies are overwhelmed by high numbers of these bodies ("excessive" apoptosis), apoptotic bodies are released into circulation and can be detected in blood plasma or serum (Holdenrieder et al., 2001a; Holdenrieder et al., 2001b; Holdenrieder et al., 2001c; Lichtenstein et al., 2001).

Above-average levels of apoptotic bodies in the bloodstream have been correlated with the presence of tumors and cancers. While this statement appears to

contradict the general observation that apoptotic levels are decreased in tumor and cancer cells, the statement is not absolute. Resistance to apoptosis is usually a late event in malignant progression--that is, resistance to apoptosis increases as the cancer grows and becomes metastatic. Therefore, early stage tumors can be characterized by slow overall growth, reflecting a high proliferation rate balanced by a high level of apoptosis. Even in late stage tumors with relatively low rates of apoptosis, the absolute number of apoptotic bodies can be high due to the large tumor mass.

Nucleolin and PARP-1 have been discovered to be unexpectedly convenient and reliable markers for the detection of apoptotic bodies, especially those shed into circulation. Detecting these antigens in circulation, such as in plasma or serum, correlates with levels of apoptosis that overwhelm the usual apoptotic body-clearing systems, such as macrophages and/or neighboring cells to the site of apoptosis.

The rejections of the claims under 35 U.S.C. § 102(b) over Martelli et al., and under 35 U.S.C. § 103(a) over Martelli et al. in view of U.S. Patent No. 6,350,452 to Riss, or in view of U.S. Patent No. 6,096,532 to Armstrong et al., have been obviated in part by appropriate amendment, and is traversed in part. Martelli et al. and Riss teach against quantifying either nucleolin or full-length PARP-1 to detect apoptosis.

Martelli et al. concerns the intracellular distribution of the nucleolar protein components during the apoptosis process in camptothecin-treated HL60 cells. According to this reference, the level of C23/nucleolin does not change as a consequence of apoptosis (Fig. 9; col. 1, page 276, lines 29-31). The reference describes the use of a monoclonal antibody to PARP-1 as a control marker of proteolytic degradation (p. 270, right column, lines 2-3). Martelli et al. does not correlate amounts of either nucleolin or full-length PARP-1 to detect apoptosis.

Riss is concerned with apoptosis marker antibodies and methods of use. The reference describes an antibody that is highly specific to only the cleaved 89kD PARP-1 product, rather than full-length PARP-1 (col. 1, lines 55-58 and col. 2, lines 43-54). Riss describes that an important attribute of the antibody is its failure to bind to the full-length PARP-1 protein (col. 3, lines 39-42). Riss uses the antibody against the cleaved 89kD PARP-1 product as a marker for apoptosis in cultured HL60 cells (Example 2). Riss does not correlate amounts of either nucleolin or full-length PARP-1 to detect apoptosis.

Armstrong et al. describes use of a bioreactor to grow large amounts of cells for cell therapy or tissue engineering (col. 1, lines 28-40). Armstrong et al. not correlate amounts of either nucleolin or full-length PARP-1 to detect apoptosis.

The present invention includes detecting apoptosis by quantifying either nucleolin or full-length PARP-1 to detect apoptosis. Martelli et al. teaches against using either nucleolin or full-length PARP-1 to detect apoptosis: this reference describes that levels of C23/nucleolin does not change during apoptosis, and uses PARP-1 as a control. Riss teaches against using full-length PARP-1 to detect apoptosis: the antibody described by Riss fails to bind to the full-length PARP-1. Armstrong et al. has been cited for elements of claim 41. Applicants submit that the claimed invention is neither anticipated by, nor obvious over, the applied references. Withdrawal of these grounds of rejection is respectfully requested.

The rejections of the claims 1-6, 10-16, 40, and 41 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement and the written description requirement, are respectfully traversed.

The present claims are directed to methods of detecting apoptosis that utilized antibody reagents to measure levels of antigens comprising nucleolin and/or full-length PARP-1. These antibodies are available from commercial sources (see, e.g., the specification at pages 12-13, Table IA and B) or the specification describes how to make them using methods that are routine to one of ordinary skill in the art (see, e.g., the specification at page 13, line 1 through page 14, line 20).

The specification of an application must be enabling as of the filing date: "The state of the art existing at the filing date of the application is used to determine whether a particular disclosure is enabling as of the filing date." M.P.E.P. § 2164.05(a). The commercial availability of an antibody after the filing date is irrelevant. "The specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public." M.P.E.P. § 2164(a). Withdrawal of this ground of rejection is respectfully requested.

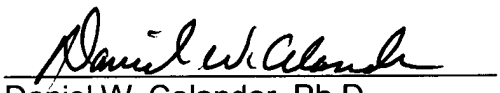
The rejections of claims 1-4, 10, 13-16, 40, and 41 under 35 U.S.C. § 112, second paragraph have been obviated by appropriate amendment.

The objection to the specification with regard to Table IA being incomplete on page 13 is respectfully traversed. The row corresponding to the antibody designated as "clone 3G4B2 mouse mAb" that begins on page 12 contains the complete description in the "Notes" column ("IgG_{1k}") for that row. Withdrawal of the objection to the specification is respectfully requested.

The objection to the drawings has been obviated by substitute drawings filed herewith.

Applicants respectfully submit that the application is in condition for allowance. Early notification of such is earnestly requested.

Respectfully submitted,



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